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Transnasal Delivery of the Peptide Agonist Specific to Neuromedin-U Receptor 2 to the Brain for the Treatment of Obesity

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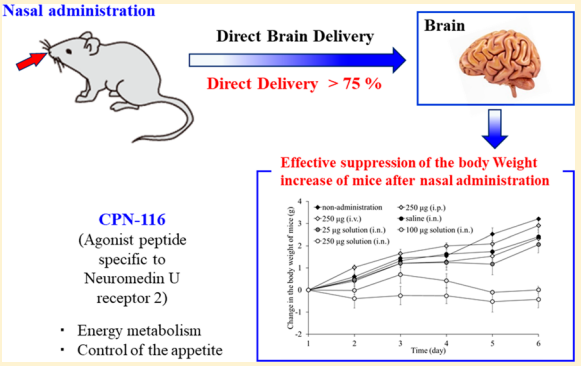
ABSTRACT: Obesity and metabolic syndrome are threats to the health of large population worldwide as they are associated with high mortality, mainly linked to cardiovascular diseases. Recently, CPN-116 (CPN), which is an agonist peptide specific to neuromedin-U receptor 2 (NMUR2) that is expressed predominantly in the brain, has been developed as a new therapeutic candidate for the treatment of obesity and metabolic syndrome. However, treatment with CPN poses a challenge due to the limited delivery of CPN to the brain. Recent studies have clarified that the direct anatomical connection of the nasal cavity with brain allows delivery of several drugs to the brain. In this study, we confirm the nasal cavity as a promising CPN delivery route to the brain for the treatment of obesity and metabolic syndrome. According to the pharmacokinetic study, the clearance of CPN from the blood was very rapid with a half-life of 3 min. *In vitro* study on its stability in the serum and cerebrospinal fluid (CSF) indicates that CPN was more stable in the CSF than in the blood. The concentration of CPN in the brain was higher after nasal administration, despite its lower concentrations in the plasma than that after intravenous administration. The study on its pharmacological potency suggests the effective suppression of increased body weight in mice in a dose-dependent manner due to the direct activation of NMUR2 by CPN. This results from the higher concentration of corticosterone in blood after nasal administration of CPN as compared to nasal application of saline. In conclusion, the above findings indicate that the nasal cavity is a promising CPN delivery route to the brain to treat obesity and metabolic syndrome.



KEYWORDS: *neuromedin U, peptide, obesity, nasal delivery, brain delivery, nose to brain*

1. INTRODUCTION

Being overweight and obesity are rapidly growing threats to the health of a large population in an increasing number of countries.[1] The World Health Organization (WHO) measures obesity on the basis of body mass index (BMI), obtained by dividing the body weight (kg) by the square of the height (m^2), and people with BMI of ≥30 are generally considered obese.[2] Obesity increases the risk of various diseases, particularly heart disease, hypertension, hyperlipidemia, and type-2 diabetes.[3] Therefore, it is one of the most serious public health problems of the 21st century. Dietary control and physical exercise are primary in the management of obesity.[4] However, when these measures fall short, medication may be essential. Commonly prescribed weight-loss medications[5] include orlistat (Xenical),[6] lorcaserin (Belviq),[7] phentermine and topiramate (Qsymia),[8] bupropion and naltrexone (Contrave),[9] and liraglutide (Saxenda).[10] However, these drugs are neither easily available nor safe to consume; in Japan, its prescription is limited to patients with BMI > 30 and

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have side effects such as diarrhea. Therefore, new therapeutic candidates with easy use and safety are highly required.

Neuromedin-U (NMU) is a neuropeptide, which was first isolated in 1985 from the spinal cord of the porcine.[11,12] NMU is believed to have many functions including appetite regulation, energy homeostasis, gastric secretion, smooth muscle contraction, bone remodeling, and in progression of types of cancer.[13−17] These are mediated through two NMU receptors (NMUR), NMUR1 and NMUR2. NMUR1 is expressed in peripheral tissues predominantly found in the gastrointestinal tract, whereas NMUR2 is expressed in the central nervous system (CNS), with the highest expression in the hypothalamus, medulla, and spinal cord.[18] It was reported that NMUR2 is involved in the energy metabolism and the control of the appetite. The food intake of rats was markedly suppressed by intracerebroventricular administration of NMU.[19] Recently, we have successfully developed CPN, which is a specific agonist peptide to NMUR2.[20] Upon activation of NMUR2 by CPN, the reduction in the body weight may likely be possible through both the reduction in the appetite and increase in energy consumption. Therefore, a specific agonist to NMUR2, such as CPN, may be a promising candidate for the treatment of obesity. However, to obtain this efficient therapeutic effect, optimized delivery of CPN to the brain is required. In general, delivery of large hydrophilic molecule peptide to the brain is difficult through the blood−brain barrier (BBB).

Recent studies have shown that some drugs directly reach the brain via the olfactory or the trigeminal nerve pathway in rats[21−23] and monkeys.[24−26] This direct connection of the nasal cavity with the brain allows delivery of many drugs, including CPN, to the brain. Additionally, the direct transport from nose to the brain can provide many advantages such as quick onset of drug action, avoidance of systemic side effects, and reduction in drug doses for the same or better therapeutic effect. The aim of this study is to evaluate the possibility of CPN delivery to the brain through the nasal cavity as well as its pharmacological potency to treat obesity.

2. MATERIALS AND METHODS

2.1. Materials. Acetonitrile, trifluoroacetic acid, and isoflurane were supplied by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Heparin sodium was purchased from nacalai tesque, Inc. (Kyoto, Japan). All other chemicals were of reagent grade and commercially available.

2.1.1. Synthesis of CPN. As reported previously,[20] the synthesis of CPN-116 was prepared using Fmoc chemistry. The pure peptide (>95% purity) collected as TFA salts through preparative RP-HPLC purification and subsequent lyophilization was treated with AG1-X8 anion exchange resins (BIO-RAD, Hercules, CA) to obtain the acetate salts for *in vivo* study.

2.2. Evaluation of Agonistic Activity to Mouse NMU Receptor 2. As reported previously,[20] the HEK293 cells maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal calf serum (FCS) were used to evaluate the agonistic activity to two mouse NMU receptors (NMUR1 and NMUR2). Cells seeded (5.0×10^5 cells per well) into 100 mm dish were transfected with respective receptor expression plasmids using FuGENE6 under the manufacturer's protocol. After transfection, the cells were seeded (4.0×10^4 cells per well) into 96-well black-wall plates with clear bottoms coated by poly-D-lysine. Eighteen hours after incubation, the cells were treated for 40 min with 4 μM Fluo-4-AM fluorescent Ca^{2+} indicator in an assay buffer (HBSS, 10 mM HEPES, 2.5 mM probenecid, pH 7.4) containing 1% FCS. After washing four times with the assay buffer without FCS, the intracellular calcium change of peptide-treated cells was measured on a fluorometric imaging plate reader (Molecular Device, Sunnyvale, CA). The peptide dissolved in an assay buffer containing 0.05% BSA and 0.001% Triton X-100 was prepared from 10^{-12}−10^{-6} M (final concentration). The maximal value of fluorescence signal was used as an efficacy of the tested peptide. EC_{50} values of agonist peptides were determined using KaleidaGraph 4.5, whose curve fit is based on Levenberg−Marquardt algorithm.

2.3. *In Vivo* Animal Study. *2.3.1. Preparation of Dosing Solutions.* For intranasal (i.n.) administration, CPN was dissolved in physiological saline at 50 mg/mL. For intravenous (i.v.) administration, the i.n. solution was diluted to 2.5 mg/mL with saline.

2.3.2. Animal Study. All animal experiments were performed according to the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication #85−23). All animal studies were approved by the Animal Ethic Committee at Kyoto Pharmaceutical University (approval number: 16−12−074) and were carried out in accordance with the guidelines provided by them. Male Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan), weighing 220−250 g (8-week-old), were used. They were caged in groups (n = 3) in a room with controlled temperature and humidity, a 12 h light−dark cycle, and with free access to water and food.

After introduction of anesthesia with intraperitoneal (i.p.) pentobarbital sodium (52 mg/kg, Somnopentyl, Kyoritsu Seiyaku Corporation, Tokyo, Japan), the right femoral artery of the rats was cannulated with polyethylene tubing for blood sampling. The CPN solution (2.5 mg/mL, 200 μL) was injected into left femoral vein at the dose of 0.5 mg/rat. CPN solution (50 mg/mL, 20 μL) was administered nasally at the dose of 1 mg/rat under the inhalation anesthesia with isoflurane. After intranasal application, the animals were kept conscious in a rat cage (KN-326-III, Natsume, Tokyo, Japan) throughout the experiment. At predetermined time intervals, blood samples were taken and then centrifuged at 12 000g for 5 min to obtain the plasma. Plasma samples were stored at −40 °C until the assay.

2.4. *In Vitro* Stability of Serum and CSF. *2.4.1. Preparation of Serum and CSF.* After rats were anesthetized with i.p. pentobarbital sodium (52 mg/kg), CSF was collected by cisternal puncture and whole blood was drawn from the polyethylene cannula inserted into the right femoral artery. Blood sample was kept at room temperature for 2 h and then stored at 4 °C overnight. On the next day, blood was centrifuged at 1200 rpm for 30 min at 4 °C and the serum was collected as supernatant.

2.4.2. Stability of CPN in Serum and CSF. CPN solution (10 μL at the concentration of 50 mg/mL in PBS) was added to 90 μL of serum. The CPN dissolved in PBS (5 μL, 50 mg/mL) was added to 15 μL of CSF. After incubation at 37 °C for a predetermined time, samples were cooled down by placing in ice. Acetonitrile (1 mL) was added to serum samples for deproteinization. Mixtures were then vortexed and centrifuged at 12 000 rpm for 5 min at 4 °C. The supernatant was transferred to tubes and evaporated to dryness. The residue was reconstituted with 100 μL of mobile phase of LC−MS

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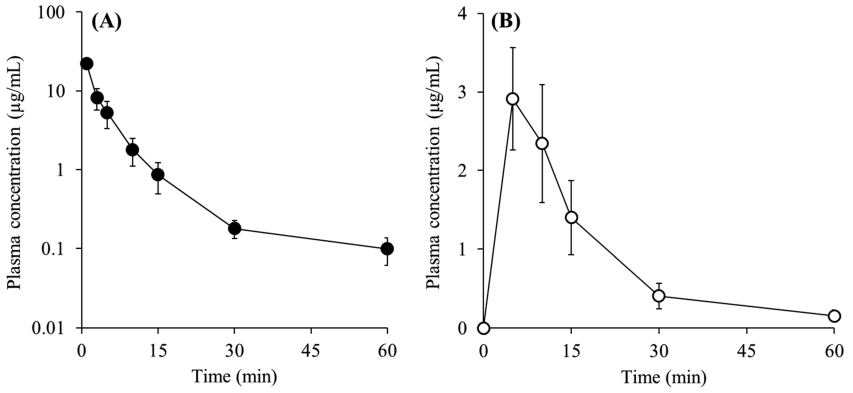

Figure 1. Profiles of CPN concentration in the plasma after (A) intravenous and (B) nasal administration. Key: ●, intravenous administration, ○, nasal administration. Results are expressed as the mean ± SE of at least three experiments.

mentioned below. The concentrations of CPN in serum were measured by LC−MS. CPN in the CSF samples was measured by LC−MS without any treatments.

2.5. *In Vivo* Brain Distribution. *2.5.1. Preparation of Dosing Solutions.* For i.n. administration, CPN was dissolved in physiological saline at the concentration of 40 mg/mL. For intravenous and i.p. administration, the i.n. solution was diluted to 2 mg/mL.

2.5.2. Animal Study. Male ddY mice, weighting 25 g, were purchased from Japan SLC (Shizuoka, Japan). Animals were maintained under conventional housing conditions. The CPN solution (5 μL) was administered nasally via left nostril of the mice at the dose of 200 μg using a micropipette as reported previously,[27] and CPN solution (100 μL) was administered intravenously or intraperitoneally at the same dose of i.n. administration. Blood was collected from the postcaval vein 5 min after administration. Then saline including heparin was flushed by perfusion from the left cardio ventricle to remove the blood from the cerebral blood vessel and the whole brain was taken.

The dissected brain was washed with ice-cold saline and divided into three sections of the brain: olfactory bulb, and frontal and occipital halves. Each brain section was weighed and homogenized in 100 μL of saline. Then 0.04 N HCl (560 μL) and saline (420 μL) were added to the homogenate. The mixture was vortexed and centrifuged. For the purification, the cartridge for solid extraction (ISOLUTE MFC 18, 100 mg, 3 mL, Biotage Japan, Tokyo, Japan) was used. The cartridge was preconditioned with 4 mL of methanol, 2 mL of 60% acetonitrile/0.1% TFA, 2 mL of 0.1% TFA, and 2 mL of saline. Samples were carefully loaded into the cartridge. The cartridge was washed with 4 mL of saline and 4 mL of 10% acetonitrile/0.1% TFA. CPN was eluted with 2 mL of 60% acetonitrile/0.1% TFA. The collected eluate was evaporated to dryness and the residue was reconstituted with 100 μL of the mobile phase of LC−MS. Pretreatment of plasma was the same as mentioned above. The concentrations of CPN in the plasma and brain were determined with LC−MS.

2.5.3. Calculation of Direct Transport Percentage. To calculate direct transport percentage (*DTP*), the equation reported by Zhang et al.[28] was slightly modified. The modified equation is as follows;

$$DTP = \frac{C_{\text{brain,i.n.}} - C_{\text{brain,i.p}} \frac{AUC_{\text{plasma,i.n}}}{AUC_{\text{plasma,i.p}}}}{C_{\text{brain,i.n.}}}$$

$C_{\text{brain, i.n.}}$ and $C_{\text{brain, i.p.}}$ are brain concentrations after i.n. and i.p. application, respectively. $AUC_{\text{plasma, i.n.}}$ and $AUC_{\text{plasma, i.p.}}$ are the areas under the curves (*AUC*) of the plasma concentration of CPN after i.n. and i.p. application. $C_{\text{brain, i.n.}}$ and $C_{\text{brain, i.p.}}$ are used in place of $AUC_{\text{brain, i.n.}}$ and $AUC_{\text{brain, i.p.}}$ of the equation reported by Zhang et al.[28] On the basis of *DTP*, the contribution of the direct nose−brain pathway to the total brain uptake of CPN after i.n. administration can be evaluated. AUC_{plasma} was calculated according to the trapezoidal rule.

2.6. *In Vivo* Pharmacological Potency. *2.6.1. Preparation of Dosing Solutions.* CPN was dissolved in physiological saline at the concentration of 50 mg/mL (250 μg/mouse). The solution of 50 mg/mL was diluted to 20 mg/mL (dose: 100 μg/mouse) and 5 mg/mL (dose: 25 μg/mouse). Diluted i.n. dosing solution (20 mg/mL) was used for i.v. and i.p. administration.

2.6.2. In Vivo Pharmacological Study. The ddY mice weighing 25 g, purchased from Japan SLC (Shizuoka, Japan), were used. Mice received CPN solution nasally (5 μL), intravenously (100 μL), and intraperitoneally (100 μL) twice every 3 days. Mice received three nasal doses (25 μg, 100 μg, 250 μg) for the evaluation of dose-dependency. The body weight and food intake were measured daily. Food intake of individual mouse could not be determined as 4−6 mice were housed in each cage.

2.7. Effect of CPN on Level of Corticosterone in Plasma. *2.7.1. Preparation of Dosing Solutions.* For i.n. administration, CPN was dissolved in physiological saline at the concentration of 50 mg/mL (250 μg/mouse). For i.p. administration, the solution of 50 mg/mL was diluted to 2.5 mg/mL (250 μg/mL).

2.7.2. Application of CPN and Sampling of Blood. The solution of CPN was administered nasally (5 μL) or intraperitoneally (100 μL) to mice. As the negative control, the vehicle (physiological saline) was applied nasally or peritoneally to mice. Blood was collected 5 min after administration of CPN.

2.8. Assay of CPN and Corticosterone. Concentrations of CPN in the plasma and brain and corticosterone (CCS) in the plasma were determined by a liquid chromatography−mass spectrometry (LC-20A and LCMS-2020, SHIMADZU, Kyoto, Japan). A C18 analytical column (TSKgel ODS 100 V, 3 μm, 2.0 mm × 100 mm, TOSOH, Tokyo, Japan) was used for chromatographic separation. The mobile phase was acetonitrile: 0.1% TFA = 33/67 (v/v) at the flow rate of 0.2 mL/min. CPN and CCS eluting from the column was detected by MS

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under the positive mode. Other conditions such as temperatures and voltages were default setting. Nitrogen gas was used for nebulization at the flow rate of 1.5 L/min. The peak area of CPN and CCS was correlated well with the concentration over wide range of CPN (2 ng/mL−10 μg/mL) and CCS (10 ng/mL−5 μg/mL), with r^2 higher than 0.998, indicating that the concentration within this range can be precisely determined.

2.9. Calculation of Pharmacokinetic Parameters of CPN. A nonlinear least-squares regression software, WinNonlin package (Pharsight Corporation, Mountain View, California, USA), was used for the analysis on plasma concentration−time profiles of CPN following i.v. application. According to the Akaike's information criterion (AIC),[29] a two-compartment model was the most preferred. Regarding the analysis on the data from i.n. application study, model-independent analysis, moment analysis, was employed to calculate *AUC* and mean residence time (*MRT*). Bioavailability (*F*) was calculated as the ratio of $AUC_{i.n.}$ to $AUC_{i.v.}$.

2.10. Data Analysis. All experiments were performed at least in triplicate, and the data are expressed as the mean ± standard error (S.E.). Statistical significance was checked based on Dunnett's test.

3. RESULTS

3.1. Pharmacokinetics of CPN in Rats. Figure 1 shows the profiles of CPN concentration in the plasma after i.n. and i.v. administration. The initial concentration of CPN after i.v. administration was 22.3 μg/mL, which then decreased rapidly. After i.n. administration, CPN concentration reached C_{max} of 2.91 μg/mL at 5 min. Table 1 lists the pharmacokinetic parameters. The *AUC*, *MRT*, total body clearance (*CL*), and the volume of distribution of CPN in the central compartment (V_d) after i.v. administration were 115.2 ± 27.7 min μg/mL, 3.47 ± 0.68 min, 5.04 ± 1.48 mL/min, 15.5 ± 0.94 mL, respectively, while *AUC* and *MRT* after i.n. administration were 55.8 ± 12.9 min μg/mL, 20.8 ± 4.3 min, respectively, and the bioavailability (*F*) was 24.2%.

Table 1. Pharmacokinetic Parameters of CPN after Intravenous and Nasal Administration[a]

	i.v.	i.n.
AUC (min μg/mL)	115.2 ± 27.7	55.8 ± 12.9
MRT (min)	3.47 ± 0.68	20.8 ± 4.3
CL (mL/min)	5.04 ± 1.48	
V_d (mL)	15.5 ± 0.94	
F (%)		24.2 ± 5.6

[a]Results are expressed as the mean ± SE of at least three experiments. Key: *AUC*, area under the curve of plasma concentration; *MRT*, mean residence time; *CL*, total body clearance; V_d, volume of distribution of the central compartment; *F*, bioavailability.

3.2. *In Vitro* Stability of CPN in Serum and CSF. Figure 2 shows the change in CPN concentration in the serum and CSF as a function of time. The serum concentration of CPN rapidly decreased to 20% of the initial concentration at 30 min, and no CPN was detected 2 h after the start of incubation. On the contrary, the decrease in the CSF concentration of CPN was gradual. The concentration in the CSF was 90% at 30 min and 20% at 4 h after the start of incubation. These findings indicated that the stability of CPN in the CSF is better than that in the serum.

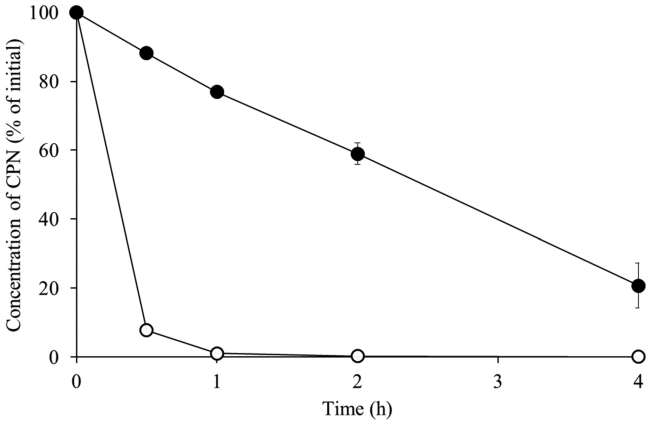

Figure 2. Change in the concentration of CPN in the serum and CSF as a function of time Key: ○, in the serum; ●, in the CSF. Results are expressed as the mean ± SE of at least three experiments.

3.3. *In Vivo* Brain Distribution in Mice. Figure 3A shows the concentration of CPN in the plasma 5 min after its i.n., i.v., and i.p. administration. The concentrations were 2.48 ± 0.30 μg/mL, 25.9 ± 1.1 μg/mL, and 7.92 ± 1.35 μg/mL, respectively. The concentration after i.n. application was the lowest. Figure 3B shows the brain concentration of CPN 5 min after application. The concentrations in the olfactory bulb were 1.13 ± 0.32 μg/mL (i.n.), 0.23 ± 0.07 μg/mL (i.v.), and 0.17 ± 0.08 μg/mL (i.p.). Brain concentrations of CPN after i.n. application were higher than those after i.v. and i.p. administration, while the plasma concentration after i.n. application was much lower as compared to that after i.v. and i.p. administration. It is also notable that the concentration in the olfactory bulb was the highest as compared to that in the frontal and occipital half of the brain.

The *DTP* of each section of the brain was higher than 70%. Particularly, the olfactory bulb showed the highest DTP of 96.1 ± 1.0%, indicating that almost all of CPN in the olfactory bulb was delivered directly from the nasal cavity.

3.4. *In Vivo* Pharmacological Potency (Change in Body Weight and Food Intake). Before *in vivo* pharmacological study using mice, the dose-dependent agonistic activities of CPN to mouse NMU receptors were evaluated. The EC_{50} values of CPN to mouse NMUR2 were calculated using KaleidaGraph 4.5. CPN exhibits a selective agonistic activity to mouse NMUR2 with an EC_{50} value of 28 ± 2 nM (means ± SD), whereas the corresponding activity of human NMU (hNMU) was 0.53 ± 0.06 nM (means ± SD) (Figure 4). As with the reactivity to hNMU receptors,[20] CPN did not show any agonistic activity to mouse NMUR1 below a concentration of 10^{-6} M (Figure 4). These results indicated that CPN selectively activates mouse NMUR2.

Figure 5A shows the change in the body weight of mice after i.n., i.v., and i.p. administration of CPN. The increase in the body weight is significantly inhibited by i.n. administration as compared to that by i.v. and i.p. administration. The CPN elicited the body weight loss of 2.1 g at the effective dose of 100 μg. The body weight loss by nasal CPN for 6 days was dose dependent at 100 μg/mouse and 250 μg/mouse. Figure 5B shows the change in the food intake. Nasal administration CPN suppressed the food intake as compared to that by i.p. and i.v. administration.

3.5. Change in Corticosterone Level in Plasma by Administration of CPN. Figure 6A and B show the plasma

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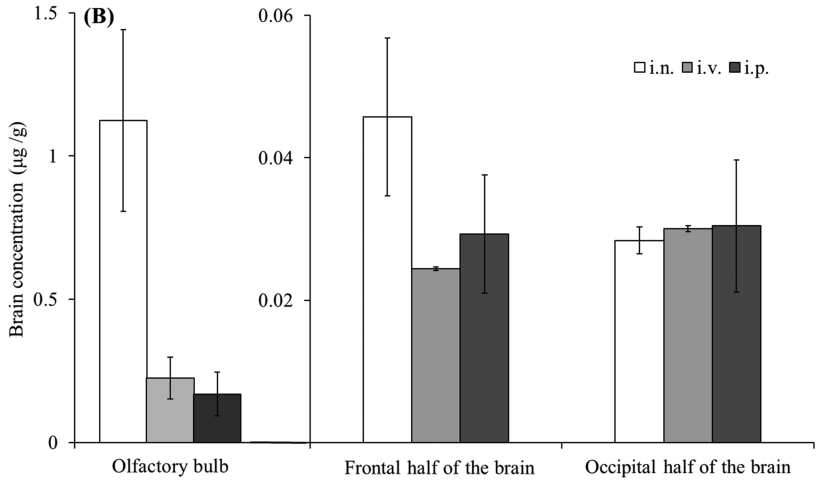

Figure 3. Concentration of CPN in the (A) plasma and (B) brain 5 min after nasal, intravenous, and intraperitoneal administration in mice. $**p < 0.01$, $* p < 0.05$ versus i.n. Key: white, i.n.; gray, i.v.; black, i.p. Results are expressed as the mean ± SE of at least three experiments.

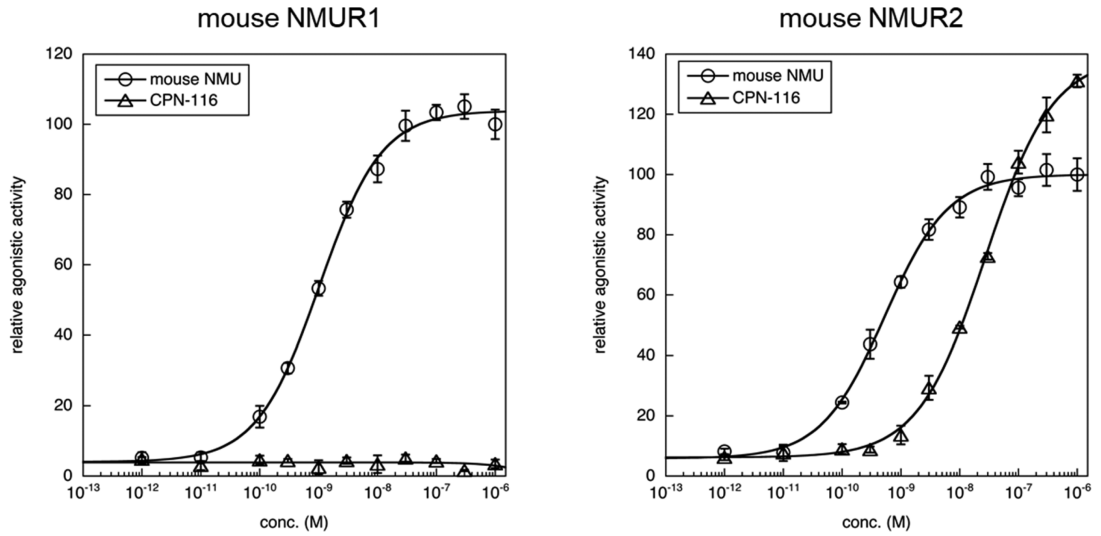

Figure 4. *In vitro* agonistic activity in HEK293 cells of CPN toward transiently expressed mouse (A) NMUR1 and (B) NMUR2 as determined by the calcium mobilization assay. Peptide concentrations, 10^{-12}–10^{-6} M; reference compound, mouse NMU. Data (means ± SD) were determined in triplicate. Curve fitting was performed using KaleidaGraph 4.5.

concentration of CPN and CCS, respectively, 5 min after i.n. and i.p. administration of CPN. The plasma concentrations of CPN were 6.49 ± 1.27 μg/mL (i.n.) and 6.13 μg/mL (i.p.). No significant difference was observed in the concentrations of CPN. The concentration of CCS in the plasma under physiological condition was 0.24 ± 0.04 μg/mL. The concentrations of CCS were 0.65 ± 0.04 μg/mL and 0.99 ± 0.05 μg/mL, respectively, after i.n. administration of saline and CPN, indicating that the nasal CPN administration increased the plasma CCS concentration. On the contrary, the concentration of CCS after i.p. administration of saline and CPN were 0.88 ± 0.09 μg/mL and 0.77 ± 0.10 μg/mL, respectively. No increase in the CCS concentration was observed after i.p. administration of CPN.

4. DISCUSSION

In general, lipophilic drugs with molecular size less than 500 Da can be transported to the brain through the BBB. However, the delivery of the peptide to the brain after its i.v. administration is exceedingly difficult because of high hydrophilicity and large molecular size.[27] One of the strategies to deliver peptides to the brain is the use of the transcytosis receptor expressed at cerebral endothelial cells. The therapeutic peptide is coupled with the antibody against transcytosis receptor for peptides such as insulin[30] and transferrin.[31] After intravenous administration, the conjugate can cross the BBB by transcytosis and interact with the receptor to exhibit the pharmacological potency, usually after the cleavage into the peptide and the antibody.

Recently, nasal administration has drawn much attention as an alternative pathway to bypass the BBB and to target drugs to the central nervous system. Direct nasal delivery has many advantages and no chemical reaction is required to prepare the conjugates. Furthermore, repeated nasal administration is possible as it is easy and noninvasive. It has been reported that some peptides, such as thyrotropin releasing hormone (TRH),[32] erythropoietin,[33] interferon-β,[24] and VEGF,[34] are directly transported to the brain through the nasal cavity. Two pathways, the olfactory and the trigeminal nerve pathways, are assumed to directly transport the drug to the brain. The

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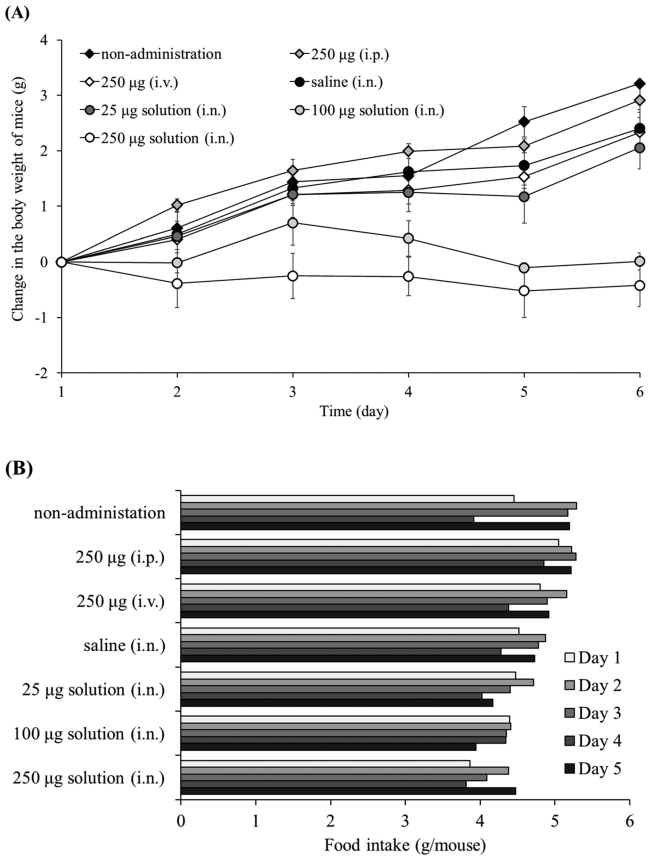

Figure 5. (A) Change in the body weight and (B) food intake of mice after nasal, intravenous, and intraperitoneal administration Results are expressed as the mean ± SE of at least three experiments.

delivery to the cerebral cortex and the olfactory bulb is feasible through the olfactory nerve pathway, while delivery to the pons and the cerebellum is feasible through the trigeminal pathway since the olfactory and trigeminal nerves start from the olfactory bulb and pons, respectively. Two transport mechanisms, transepithelial and retrograde axonal transport, are involved in the olfactory nerve pathway. The mechanism of the transepithelial transport is passive diffusion. The drug is diffused through the nasal epithelium and then transported through small holes of the cribriform plate into the inside of the skull to reach the brain or subarachnoid CSF. In case of the retrograde axonal transport, the drug initially undergoes endocytosis by the olfactory sensory neuron. The axon terminal of the olfactory sensory neuron is exposed at the olfactory region in the nasal cavity. Following endocytosis, the drug undergoes retrograde axonal transport. The long nerve cell possesses a special transport mechanism to transport nutrients or other molecules intracellularly to the axon terminal of the neuron from the cell body. The intracellular drug is transported retrogradely from the axon terminal to the neuron cell body. As this process is very slow, it could take a few hours or days for the drug to reach the brain from the nasal cavity. At present, the mechanism of transport via trigeminal nerve pathway is not clearly established. Because the highest uptake of CPN was observed in the olfactory bulb, the olfactory nerve pathway is likely involved in the direct transport of CPN.

The exchange system of the fluid between the CSF and brain extracellular fluid (BECF) has been deciphered recently.[35,36] This system is called glymphatic system. The subarachnoid CSF enters the brain deeply along the arterial blood vessel (para-arterial influx) and BECF exits out of the brain back to the subarachnoid space along the venous blood vessel (para-venous clearance). According to Iliff et al., glymphatic system is likely responsible for the removal of waste with the large-size such as amyloidβ (Aβ) protein.[37] The glymphatic system is activated during the sleep. The elimination of Aβ is enhanced to prevent Alzheimer disease. The glymphatic system may allow the wider distribution of the drug directly delivered to CSF.

Disposition and nasal absorption of CPN were examined. The clearance of CPN from the blood after i.v. bolus administration was rapid with the initial half-life of a few minutes, resulting in the large *CL* value of 5 mL/min. Additionally, CPN was degraded rapidly in the serum *in vitro*. The degradation of CPN in the serum markedly contributed to the rapid plasma clearance of CPN. The concentrations of CPN after nasal administration were high. The bioavailability of CPN was 24.2%, which was better than that estimated from the molecular size and hydrophilicity of CPN.

The stability study shows that CPN undergoes rapid degradation in the serum with the half-life of less than 10 min, whereas CPN was relatively stable in the CSF. According to our preliminary study, thrombin is involved in the degradation of CPN in the serum. These results suggested that the direct delivery of CPN, which avoids degradation in

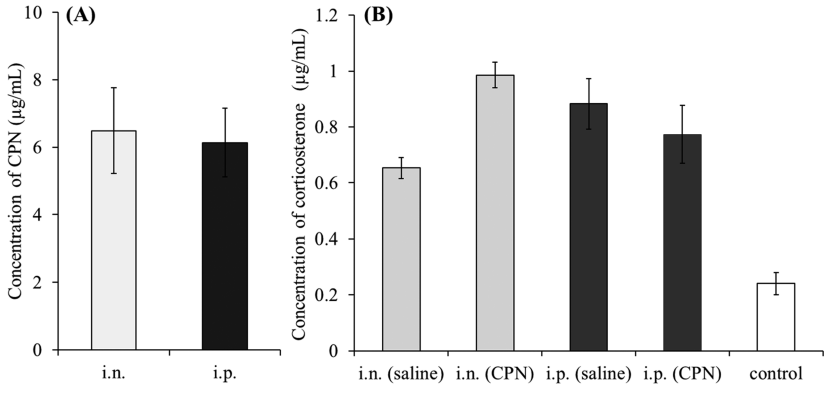

Figure 6. Concentrations of (A) CPN and (B) CCS in the plasma 5 min after nasal and intraperitoneal administration of CPN Results are expressed as the mean ± SE of at least three experiments.

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the blood, would be a favorable approach for the efficient delivery of CPN to the brain.

The pharmacokinetic analysis has clarified that the brain concentrations of CPN following i.n. application were higher than those after i.p. and i.v. application, despite the lower plasma concentrations following i.n. application. It is notable that the concentration of CPN in the olfactory bulb, which is located most closely to the nasal cavity, was highest among the brain regions. These results indicated that the efficient brain delivery of CPN was achieved successfully by nasal application. To evaluate the contribution of nose-to-brain direct delivery, the total brain uptake of CPN and DTP was calculated based on the pharmacokinetic data. The DTP after nasal administration was greater than 70%. These results indicate that direct delivery of CPN was predominant over the normal pathway through BBB. The rank order of DTP is the olfactory bulb > the frontal half > the occipital half, indicating that the DTP was higher in the brain region close to the nasal cavity.

Pharmacological study demonstrated that nasally applied CPN significantly inhibited the food intake and the increase in the body weight of mice in a dose dependent manner. These results are in good agreement with those on the brain uptake of CPN. To make sure that CPN has activated NMUR2, the blood concentration of CCS was measured after i.n. and i.p. administration. CCS is a hormone released from the adrenal gland. When CPN activates NMUR2 in the hypothalamus, corticotropin releasing hormone (CRH) is secreted, stimulating the release of adrenocorticotropic hormone (ACTH) from the pituitary gland.[38] The ACTH stimulates the adrenal gland to secrete CCS into the blood. Therefore, the concentration of CCS in the blood is considered as an indicator of the direct activation of NMUR2 by CPN. The concentration of CCS after i.p. administration of CPN was similar to that of positive control, indicating that CPN failed to reach the brain. In contrast, the CCS concentration after i.n. administration was significantly higher than that of the positive control group, despite the similar blood concentration of CPN with that after i.p. administration. These results suggested that the inhibition of body weight increase and food intake is mediated by the activation of NMUR2 by CPN after direct delivery to the brain by i.n. administration.

In our previous study,[27] the plasma pharmacokinetics and the delivery of oxytocin to the brain after i.n. administration has been described. Molecular sizes of both peptides are similar (CPN: 807 Da, Oxytocin: 1001 Da), while the stability in the plasma/CSF and the nasal absorption (transepithelial permeability) are different. The stability of CPN in the serum/CSF was poor, but the nasal absorption of CPN was better than that of oxytocin. The concentrations of oxytocin in the plasma and in the olfactory bulb 5 min after i.n. administration to mice at the dose of 100 μg were 0.19 ± 0.01 μg/mL and 0.70 ± 0.27 μg/g tissue, respectively, while those of CPN at the dose of 200 μg were 2.48 ± 0.30 μg/mL and 1.13 ± 0.32 μg/g tissue. The concentration in the plasma and the brain is higher despite its lower dose. Oxytocin showed DTP larger than 95%, which was much higher than that of CPN. The higher plasma concentrations and the better transendothelial permeation of CPN may have rendered its higher uptake to the brain from the blood, through BBB. The positive charge of CPN may have resulted in its permeation across nasal epithelial cells and the BBB; however, the mechanism for this is not yet clear.

5. CONCLUSIONS

In conclusion, the direct delivery of CPN to the brain was successfully achieved by i.n. administration. Thus, the i.n. administration is a promising approach for the efficient delivery of CPN to the brain for the treatment of obesity.

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Notes

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